Reproducibility and Comparability of Insulin Sensitivity Indices Measured by Stable-label Intravenous Glucose Tolerance Test

R. Hovorka[1]*, P. Bannister[3], D.J.A. Eckland[2], D. Halliday[3], D.N. Murley[1], S.E. Rees[1], M.A. Young[2]

[1]Metabolic Modelling Group, Centre for Measurement and Information in Medicine, City University, London, UK
[2]Glaxo Wellcome Research and Development plc, Greenford, Middlesex, UK
[3]MRC Human Metabolism Research Group, Unit of Metabolic Medicine, St Mary's Hospital Medical School, London, UK

We have investigated the reproducibility of (1) insulin sensitivity (S^*_I) and glucose effectiveness (S^*_G) as measured by the stable-label (one compartment) minimal model, and (2) insulin sensitivity (S^*_{Ib}), plasma clearance rate (*PCR*), basal hepatic output (HGO_b), and total hepatic glucose output (HGO_{0-240}) as measured by the novel stable-label two compartment model of glucose disappearance during labelled intravenous glucose tolerance test (IVGTT) using 6,6-^{2}H-glucose. Ten normal male subjects were studied on two occasions one week apart. Both models provided estimates of all indices with acceptable precision (CV of parameter estimates $\leq$50 %). The within subject CVs of S^*_I and S^*_{Ib} were comparable (17 % vs 19 %) as were the within subject CVs of S^*_G and PCR (13 % vs 16 %). A highly significant linear relationship was observed between S^*_{Ib} and S^*_I (0.303 ± 0.046 ml kg^{-1} min^{-1} per mU l^{-1} vs 13.04 ± 1.89 10^{-4} min^{-1} per mU l^{-1}, $y = 0.0037\ x + 0.0002$, $r = 0.90$, $p < 0.001$; mean $\pm$ SE), but not between *PCR* and S^*_G (1.98 ± 0.15 ml kg^{-1} min^{-1} vs 0.0089 ± 0.0005 min^{-1}, $r_s = 0.34$, NS). The two compartment model provided a plausible time-profile of hepatic glucose output during IVGTT, reproducible estimates of HGO_b (1.96 ± 0.18 mg kg^{-1} min^{-1}, 15 %; mean $\pm$ SE, within subject CV), and a highly reproducible HGO_{0-240} (7 %; within subject CV). We conclude that the stable-label (one compartment) minimal model and the stable-label two compartment model provide reproducible estimates of parameters of glucose kinetics in normal subjects. Insulin sensitivity indices estimated by the two models are strongly linearly related.

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Introduction

The minimal model of glucose disappearance[1] was developed to measure parameters of carbohydrate metabolism using a simpler experimental design (an intravenous glucose tolerance test or IVGTT), while employing a more sophisticated data processing, than the glucose clamp technique.[2] The minimal model provides two parameters which describe carbohydrate metabolism: insulin sensitivity which quantifies the sensitivity of tissue glucose utilization to insulin, and glucose effectiveness, which quantifies the effect of glucose levels *per se* on glucose disappearance. The need to employ a model-based approach to analyse IVGTT data is due to the dynamic nature of the glucose and insulin data and the delay in insulin action. Visual assessment of individual data sets fails to discriminate between the effect of insulin and the mass effect of glucose on the glucose curve.

Although employed in numerous studies,[3–7] it has been argued that the original minimal model '. . .approach has been used far less than might be expected from its potential value'.[8] Elaborating on the original minimal model, two different approaches have been suggested to improve its performance. First, modified IVGTT protocols have been developed using either tolbutamide injection[9] or insulin infusion[5,6] to enhance the resolution of the insulin effect on glucose disposal. Second, the labelled minimal model using either a radioactive tracer,[10]

*Correspondence to: Dr Roman Hovorka, Metabolic Modelling Group, Centre for Measurement and Information in Medicine, City University, Northampton Square, London, EC1V 0HB, UK

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or more recently a stable-isotope tracer,[8] have been developed. The former methods improve the precision of parameter estimates[11] and allow the parameters of the minimal model to be estimated in subjects without endogenous insulin secretion (the insulin-modified IVGTT protocol) but they do not separate glucose disposal from glucose production. The latter methods separate the two processes and also substantially improve the precision of parameter estimates.[8,10]

Recently, a new stable-isotope tracer ('stable-label') *two compartment* model of glucose disappearance has been suggested by Caumo *et al*.[12] The central compartment represents glucose in the accessible (measurable) pool, the peripheral compartment represents glucose in the non-accessible pool. Unlike the (one compartment) minimal model, the two compartment model provides plausible estimates of hepatic glucose output during IVGTT due to the adoption of a more realistic structure of glucose kinetics.[13] It also provides estimates of insulin sensitivity and plasma clearance expressed in the same units as indices from glucose clamp studies. However, compared to the minimal model, limited information is available about the performance of the two compartment model.[14]

The reproducibility of and relationship between insulin sensitivity indices measured by the stable-label (one compartment) minimal model and the stable-label two compartment model is unknown. In the present study normal subjects have undergone stable-label IVGTT studies on two occasions one week apart employing 6,6-^{2}H-glucose as the stable-label tracer. Data were analysed using both models and also by the original unlabelled one compartment minimal model in order to address the issues of reproducibility and comparability providing novel insights.

Subjects and Methods

Subjects

Ten normal healthy male subjects (age: 33 ± 3 years, body weight: 79 ± 2 kg, BMI: 24.1 ± 0.4 kg m^{-2}; mean $\pm$ SE) with no family history of diabetes were studied. The subjects were admitted at about 6:00 pm prior to the study day, had a standard meal at 6:30 pm and a standard snack at 10:00 pm. They then fasted until the end of the study on the following day. Water was permitted as required. The study was approved by the Harrow Health Authority Ethical Review Committee.

Protocol

Each subject underwent two identical study days one week apart. No medication was given and the subjects followed their standard diet regimen and refrained from strenuous exercise during the week prior to the first study day and during the period between the two study days.

At about 8:00 am on each study day, a sampling cannula was inserted retrogradely in a hand vein and the hand was placed in a hot box (55 °C air temperature) to arterialize blood samples. The cannula was kept patent with heparinized saline. In the contralateral arm, a cannula was inserted in an ante-cubital vein for the administration of the bolus of isotopically labelled glucose.

After a rest period of approximately 30 min, baseline blood samples for the measurement of insulin and glucose were taken at –10 min, –5 min, and immediately prior to the administration of the glucose bolus. The bolus of labelled glucose was administered over 60 s and blood samples were taken at the following times relative to the beginning of the bolus injection: 2, 3, 4, 5, 8, 10, 12, 14, 16, 18, 20, 24, 28, 32, 40, 45, 50, 60, 70, 80, 90, 100, 110, 120, 140, 160, 180, 210, and 240 min. All samples were analysed for insulin, glucose, and 6,6-^{2}H-glucose.

Glucose Bolus

Isotopically labelled 6,6-^{2}H-glucose (98 % purity) was obtained from Cambridge Isotope Laboratories, Massachusetts, USA. The 50 % dextrose solution enriched with 13.0 ± 0.5 % of 6,6-^{2}H-glucose was given as a bolus of 0.25 g kg^{-1} body weight.

Mass Spectroscopic Analysis

Plasma samples were derivatized as the butylboronic acid acetates by the method of Weicko *et al*.[15] A Varian 3400/Finningan 4500 GC-MS equipped with a split/splitless injector, a CTC A200S autosampler and an INCOS data system were used to determine in duplicate the isotope ratios of 6,6-^{2}H-glucose. The following fragment ions were monitored: m/z 297 (^{12}C-glucose) and 299 (6,6-^{2}H-glucose). Samples from a single subject were run in one batch to eliminate the effect of the inter-assay measurement error on the reproducibility.

Insulin and Glucose Assay

Plasma insulin was measured by radioimmunoassay at Serono Diagnostics Laboratories, Welwyn Garden City, UK. The intra-assay CV was 6 % for insulin assay. Plasma glucose was determined on an IL Monarch 2000 autoanalyser (the intra-assay CV $\leq$ 1 %).

Data Analysis

6,6-^{2}H-Glucose Concentration

The concentration of 6,6-^{2}H-glucose tracer (tracer glucose) is not directly measured by the GC-MS analysis but the concentration can be calculated from tracer-to-tracee ratio (TTR).[8,16] The concentration of the 6,6-^{2}H-glucose (labelled glucose) $g^*(t)$ was obtained as

$$g^*(t) = g(t)\,\frac{z(t)}{1 + z(t)} \qquad (1)$$

where $g(t)$ is the (total) plasma glucose concentration measured by the glucose assay, and $z(t)$ is the TTR of 6,6-^{2}H-glucose. TTR was calculated from the raw measurement $r(t)$, i.e. the isotope ratio M+2/M+0 in the mixture of tracer and tracee (sample), and the raw basal measurement r_N, i.e. the isotope ratio M+2/M+0 of the most abundant isotopemer in 6,6-^{2}H-glucose to the most abundant isotopemer in the endogenous glucose, according to

$$z(t) = \frac{r(t) - r_N}{0.9661 - 0.0673\,r(t)} \qquad (2)$$

Derivation of equation (2) is described in the Appendix together with other technical details related to the description of glucose models and calculation techniques. Figure 1 shows the three models of glucose kinetics.

Unlabelled Minimal Model of Glucose Kinetics (Unlabelled One Compartment Model)

The unlabelled minimal model of glucose disappearance[1] after an IVGTT employs a single compartment to describe glucose kinetics. The model provides two indices, namely insulin sensitivity S_I and glucose effectiveness S_G. S_I represents the increase in net fractional glucose clearance per unit change in plasma insulin concentration following the intravenous glucose load. S_G represents the net fractional glucose clearance at the basal glucose and insulin concentrations. S_G measures the ability of glucose to promote its own disposal and to inhibit its own production when insulin is basal.

Importantly both S_I and S_G include two components, an acceleration of glucose disposal in tissues and an inhibition of hepatic glucose output.

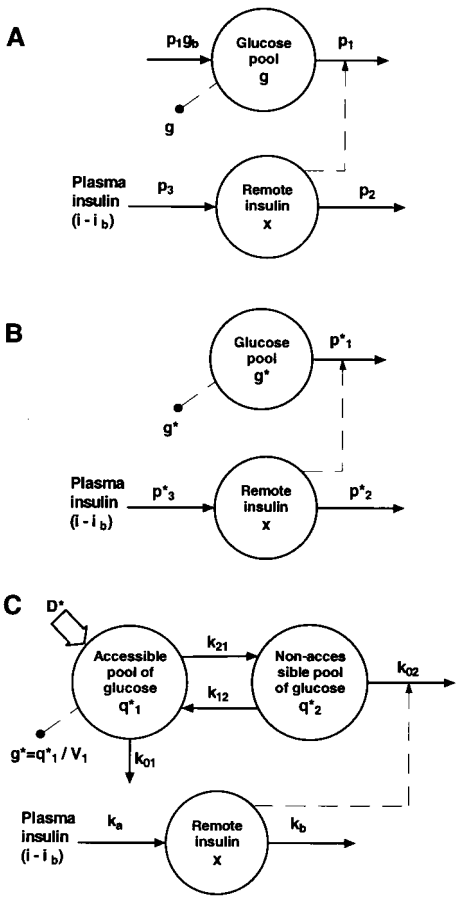

Figure 1. Models of glucose kinetics: (A) the unlabelled minimal model, (B) the stable-label minimal model, and (C) the stable-label two compartment model. A detailed description of model quantities is given in the Appendix

Stable-Label Minimal Model of Glucose Kinetics (Labelled One Compartment Model)

The stable-label minimal model of glucose disappearance[8] after an IVGTT also employs one compartment to describe glucose kinetics. Two indices are provided by the model, insulin sensitivity S^*_I and glucose effectiveness S^*_G.

S^*_I and S^*_G are estimated by fitting the model to 6,6-^{2}H-glucose concentration. Unlike unlabelled glucose, 6,6-^{2}H-glucose is not produced endogenously and S^*_I and S^*_G therefore reflect solely the promotion of glucose disposal in tissues without including the component measuring the inhibition of hepatic glucose output. Because S^*_I and S^*_G measure only disposal processes, S^*_I and S^*_G should be less than the corresponding values S_I and S_G.[8]

Stable-Label Two Compartment Model of Glucose Kinetics (Labelled Two Compartment Model)

The stable-label two compartment model of glucose disappearance during an IVGTT[12] describes glucose kinetics employing two compartments. The central compartment represents glucose in the accessible (measurable) pool, the peripheral compartment represents glucose in the non-accessible pool.

The model provides two indices of glucose kinetics, insulin sensitivity index S^*_{Ib} and plasma clearance rate *PCR*. S^*_{Ib} represents the change in plasma glucose clearance rate per unit change in plasma insulin concentration. *PCR* represents plasma clearance rate at basal glucose and insulin concentrations. The indices are estimated from the kinetics of 6,6-^{2}H-glucose and thus reflect only disposal processes.

When combined with the measurement of basal glucose, the model provides an estimate of basal hepatic glucose output HGO_b. HGO_b is the glucose appearance rate which maintains glucose concentration at the basal level given the calculated plasma clearance rate.

Parameter Estimation

The model parameters to be estimated are (1) p_1, p_2, p_3, and g_0 for the unlabelled one compartment model, (2) p^*_1, p^*_2, p^*_3, and g^*_0 for the labelled one compartment model, and (3) V_1, k_p, k_{21}, k_{12}, k_{02}, k_a and k_b for the labelled two compartment model. The parameter estimation procedure employed the plasma insulin concentration as the model input and as the output the unlabelled or labelled glucose plasma concentration for the unlabelled and labelled models, respectively. The labelled two compartment model also used the total glucose concentration as the model input. The parameter estimation procedure provided the precision of a parameter estimate expressed as the CV of the parameter estimate[17] and precision of derived parameters S_I, S_G, S^*_I, S^*_G, S^*_{Ib}, *PCR*, and HGO_b.

A weighted non-linear regression analysis was employed to estimate the parameters. The weight was defined as the reciprocal of the square of the measurement error.[17] The coefficient of variation (CV) of the measurement error of 6,6-^{2}H-glucose was determined using regression analysis from the duplicate measurements of the isotope ratio as $CV(\%) = 12.30\, e^{-15.90\, x} + 1.64\, e^{-0.83\, x}$, where x is the concentration of the tracer glucose in the plasma. The CV of the measurement error of unlabelled glucose was assumed at the level of 3 %. The plasma concentration of the labelled and unlabelled glucose was zero weighted from 2 to 5 min to estimate parameters of the unlabelled and labelled one compartment models. All measured labelled glucose concentrations were employed in the parameter estimation of the labelled two compartment model.

Hepatic Glucose Output

Hepatic glucose output was estimated using the labelled two compartment model. First, the plasma glucose component due to hepatic glucose output (endogenous glucose component) was calculated. The endogenous glucose component represents plasma concentration due to endogenous glucose production. Importantly, the endogenous glucose component is calculated directly from the measurements of total glucose, labelled glucose, and the enrichment of the glucose bolus without postulating any model in the calculations.[18,19] These so-called model independent calculations are made possible due to 6,6-^{2}H-glucose being injected in a known ratio with the unlabelled exogenous glucose. This allows the contribution of exogenous glucose to the total glucose concentration to be calculated from the concentration of 6,6-^{2}H-glucose.

Hepatic glucose output was calculated as the glucose appearance rate which achieved the glucose concentration identical to the endogenous glucose component. The endogenous glucose component is non-constant during IVGTT. Similarly the plasma clearance rate and the glucose kinetics as described by the labelled two compartment model are non-constant (time-variant) due to variable insulin and glucose concentrations during IVGTT. The calculations take into account these dynamic processes. Smoothing is required as the calculations are sensitive to the measurement error present in the endogenous glucose component.

Cumulative hepatic glucose output $HGO_{0–240}$ (total *HGO* during 0–240 min) was calculated as an index of hepatic glucose output during IVGTT.

Statistical Analysis

The reproducibility of insulin sensitivity and hepatic glucose output indices were assessed separately. In each case indices were analysed by ANOVA allowing for effects due to subject. This gave an estimate of the within subject reproducibility of the duplicate responses (within subject CV, within subject variation as % of total variation, and 95 % range for difference between duplicate measurements in one individual.[20] A comparison of the two estimates of insulin sensitivity S^*_I and S^*_{Ib} was made by analysing the mean values of the duplicate measurements using linear regression analysis and Pearson correlation coefficient. Glucose effectiveness S^*_G and plasma clearance rate *PCR* were compared using Spearman correlation coefficient to account for skewed distribution of *PCR* and S^*_G. The values are represented as mean ± SE unless stated otherwise.

Results

Total Glucose, 6,6-^{2}H-Glucose, and Insulin

The mean basal plasma glucose was 5.5 ± 0.1 mmol l^{-1}, the mean basal plasma insulin 5.2 ± 0.4 mU l^{-1}. The time profile of total glucose, 6,6-^{2}H-glucose, and insulin are shown in Figure 2. The three panels show the mean profiles from the two study days. After the administration of the glucose bolus, the mean plasma glucose and the mean plasma insulin returned to basal value within 90 min. As expected 6,6-^{2}H-glucose continued to decrease during the whole experiment.

Estimates and Precision of Indices from One Compartment and Two Compartment Models of Glucose Kinetics

Estimates of insulin sensitivity and glucose effectiveness based on the one compartment model, and estimates of insulin sensitivity, plasma clearance rate, and fasting hepatic glucose output based on the two compartment model IVGTT are shown in Table 1. The labelled models provided estimates of all indices with acceptable precision. The CV of parameter estimates was <15 % with five exceptions (subject 5, day 1: S^*_{Ib}; subject 8, day 1: S^*_{Ib}; subject 10, day 7: S^*_{Ib}, *PCR* and HGO_b) all of which were ≤ 50 %. Parameters p^*_2 (0.0129, 0.0061–

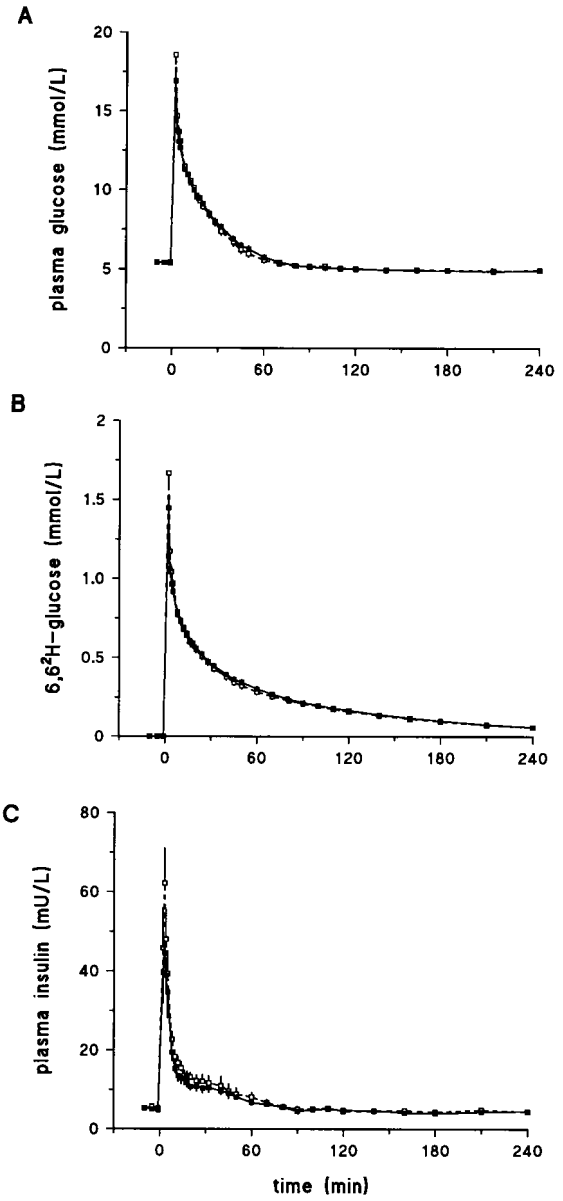

Figure 2. (A) Plasma glucose, (B) 6,6-^{2}H-glucose, and (C) plasma insulin during the stable-label IVGTT. Mean ± SE values from the first occasion (full square) and one week later (open square) are shown

0.0216 min^{-1}; median, range) and p^*_3 (1.21, 0.54–5.67×10^{-4} min^{-2} per mU l^{-1}) were estimated with acceptable precision (17, 1–88 % vs 12, 5–57 %; median, range; CV of parameter estimate p^*_2 vs p^*_3). The median and range of k_a was 2.55, 0.46–7.66×10^{-4} min^{-2} per mU l^{-1} (CV of parameter estimate 16, 4–187 %), k_b: 0.098, 0.056–0.379 min^{-1} (19, 5–112 %), k_{21}: 0.242, 0.064–0.674 min^{-1} (14, 10–41 %), k_{12}: 0.158, 0.040–0.278 min^{-1} (9, 4–35 %), k_{02}: 0.0036, 0.0023–0.0056 min^{-1} (5, 3–16 %), V_1: 92, 35–160 ml kg^{-1} (10, 0–50 %), k_p: 0.029, –0.0028–0.0140 min^{-1}.

The unlabelled model did not provide estimates of S_I on six occasions and estimates of S_G on one occasion due to low precision (CV ≫100 %) and non-feasible values. Precision of the remaining sensitivity estimates was lower when compared to precision of corresponding indices obtained from the labelled models.

Estimates of Endogenous Glucose Component and Hepatic Glucose Output

Time profiles of the endogenous glucose component and hepatic glucose output are shown in Figure 3. The endogenous glucose component started to decrease immediately after the administration of the glucose bolus and reached a minimum of ~60 % of the fasting value at 70 min. At the end of the experiment, the endogenous glucose component returned to ~80 % of the fasting value. Hepatic glucose output reached a minimum of ~65 % of the fasting value at 50 min and returned to ~90 % of the fasting value at the end of the experiment. Individual values of basal and total hepatic glucose output are listed in Table 1.

Comparability and Reproducibility of Indices from One Compartment and Two Compartment Models

Reproducibility of insulin sensitivity and glucose effectiveness based on the unlabelled and labelled one compartment model, and reproducibility of insulin sensitivity, plasma clearance rate, fasting hepatic glucose output, and cumulative hepatic glucose output based on the two compartment model are reported in Table 2.

The reproducibility of insulin sensitivity S_I was calculated from five subjects who provided estimates of S_I with acceptable precision on both occasions. Thus reproducibility of S_I was determined with less confidence than reproducibility of remaining indices calculated from 10 subjects (reproducibility of S_G based on 9 subjects).

Reproducibility of parameters of the labelled models was better than that of the unlabelled model. Reproducibility of two insulin sensitivity indices S^*_I and S^*_{Ib} was nearly identical (within subject CV 17 % vs 19 %). Reproducibility of glucose effectiveness S^*_G and *PCR* was similar (within subject CV 13 % vs 16 %). The cumulative hepatic glucose output HGO_{0-240} demonstrated the best reproducibility (within subject CV of 7 %).

The two insulin sensitivity indices S^*_I and S^*_{Ib} were strongly linearly related ($y = 0.0037x + 0.0002$, $r = 0.90$, $p < 0.001$; Figure 4). Significant correlation was not present between S^*_G and *PCR* ($r_s = 0.34$, NS).

Discussion

Reproducibility of Insulin Sensitivity, Glucose Effectiveness, and Plasma Clearance Rate

The present study shows that the stable-label (one compartment) minimal model and the stable-label two compartment model provide reproducible estimates of

Table 1. Estimates of (1) insulin sensitivity (S^*_I and S_I) and glucose effectiveness (S^*_G and S_G) based on the labelled and unlabelled one compartment model, and (2) insulin sensitivity (S^*_{Ib}), plasma clearance rate (*PCR*), fasting hepatic glucose output (HGO_b), and cumulative hepatic glucose output ($HGO_{0–240}$) based on the labelled two compartment model of IVGTT

		One compartment model				Two compartment model			
Subject	Day	S^*_1 (10^{-4}/min per mU l^{-1})	S_I (10^{-4}/min per mU l^{-1})	S^*_G (/min)	S_G (/min)	S^*_{Ib} (ml kg^{-1} min^{-1} per mU l^{-1})	*PCR* (ml kg^{-1} min^{-1})	HGO_b (mg kg^{-1} min^{-1})	$HGO_{0–240}$ (mg kg^{-1} 240 min^{-1})
1	1	7.72 (3)[a]	6.46 (78)	0.0095 (2)	0.0166 (71)	0.185 (5)	1.91 (3)	2.02 (2)	396
	7	11.14 (3)	12.81 (13)	0.0073 (2)	0.0039 (93)	0.238 (12)	1.80 (7)	1.83 (7)	390
2	1	7.09 (6)	3.29 (148)	0.0090 (2)	0.0278 (26)	0.225 (4)	1.72 (5)	1.66 (5)	343
	7	12.52 (4)	8.70 (34)	0.0086 (2)	0.0224 (26)	0.289 (3)	1.69 (4)	1.61 (4)	342
3	1	13.59 (3)	N/A (≫100)	0.0085 (2)	0.0340 (42)	0.304 (4)	1.64 (4)	1.67 (4)	341
	7	15.9 (3)	8.24 (52)	0.0089 (3)	0.0413 (44)	0.264 (3)	1.90 (2)	1.92 (2)	400
4	1	19.06 (3)	N/A (≫ 100)	0.0103 (2)	0.0624 (10)	0.454 (3)	1.88 (3)	1.66 (3)	391
	7	18.63 (2)	N/A (≫ 100)	0.0065 (3)	0.0504 (58)	0.370 (2)	1.60 (4)	1.59 (4)	381
5	1	6.60 (5)	11.02 (6)	0.0081 (2)	0.0132 (14)	0.193 (20)	1.93 (13)	1.92 (14)	318
	7	6.32 (3)	6.19 (12)	0.0085 (2)	0.0199 (16)	0.158 (3)	1.49 (3)	1.48 (3)	292
6	1	18.93 (2)	7.13 (105)	0.0088 (2)	0.0439 (57)	0.353 (4)	1.36 (3)	1.38 (3)	372
	7	14.08 (2)	9.16 (58)	0.0095 (3)	0.0364 (77)	0.268 (3)	2.00 (3)	2.00 (3)	436
7	1	13.49 (5)	10.82 (25)	0.0095 (2)	0.0189 (26)	0.360 (3)	2.23 (3)	2.12 (3)	477
	7	9.14 (4)	N/A (≫ 100)	0.0082 (3)	0.0379 (61)	0.295 (9)	2.40 (8)	2.31 (8)	518
8	1	12.01 (5)	5.03 (35)	0.0071 (2)	0.0398 (23)	0.136 (50)	2.32 (10)	2.09 (10)	383
	7	13.1 (2)	N/A (≫ 100)	0.0053 (4)	N/A (≫ 100)	0.252 (2)	1.27 (4)	1.19 (4)	323
9	1	26.25 (7)	5.51 (90)	0.0124 (2)	0.0303 (9)	0.728 (9)	3.27 (3)	3.67 (3)	665
	7	24.02 (3)	N/A (≫ 100)	0.0129 (2)	0.0476 (12)	0.589 (4)	3.20 (2)	3.38 (2)	623
10	1	6.97 (9)	8.17 (20)	0.0096 (2)	0.0215 (15)	0.235 (6)	2.01 (3)	1.88 (3)	348
	7	4.25 (9)	8.29 (23)	0.0092 (2)	0.0144 (27)	0.172 (25)	1.91 (30)	1.81 (30)	325

[a]Numbers in parentheses are parameter precision values expressed as the coefficient of variation of a parameter estimate.
N/A, not available; parameter estimation procedure returned imprecise estimate (CV ≫ 100 %).

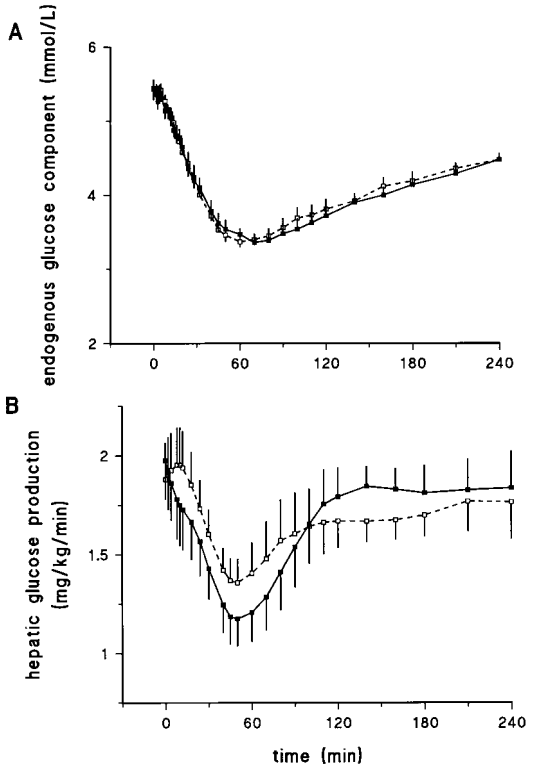

Figure 3. (A) Endogenous glucose component and (B) hepatic glucose output during the stable-label IVGTT. *HGO* was calculated using the two compartment model of glucose kinetics by employing regularization method to reconstruct an input function. Mean ± SE values from the first occasion (full square) and one week later (open square) are shown

insulin sensitivity, glucose effectiveness, plasma clearance rate, and hepatic glucose output. The results suggest that the choice of the modelling approach (one or two glucose compartments) is not influenced by issues of reproducibility. However, the two compartment model provides plausible values of the hepatic glucose output (*HGO*) and therefore needs to be employed if the measurement of *HGO* is of essence.

The study was designed to minimize the effect of external factors on the duplicate assessment of the indices. Prior to the study day the subjects had been admitted to the hospital and had a standard dinner followed by a standard evening snack. In addition, the subjects avoided strenuous physical exercise one week prior to the first study day and between the two study days as physical activity influences insulin sensitivity.[21]

Reproducibility of insulin sensitivity and glucose effectiveness from the labelled minimal model is superior to reproducibility of the indices estimated from the unlabelled *unmodified* minimal model (see Table 2). The unsatisfactory performance of the unlabelled minimal model has been long recognized. To improve the performance, tolbutamide[9,22] and insulin[6,23] modified IVGTT have been suggested and employed to provide more precise estimates of insulin sensitivity and glucose effectiveness.

For the purpose of the assessment of a drug treatment on insulin sensitivity and glucose effectiveness, the tolbutamide-modified IVGTT is not suitable due to a potential drug-tolbutamide interaction.[24] The insulin-modified IVGTT potentially influences the assessment of the second phase insulin secretion as plasma insulin concentration includes component due to the exogenous insulin. The radioactive-label IVGTT[10] is inferior to the stable-label IVGTT for ethical reasons.

The reproducibility of insulin sensitivity and glucose effectiveness provided by the *stable-label unmodified* (one compartment) minimal model is comparable to the reproducibility provided by the unlabelled *tolbutamide-modified* (one compartment) minimal model in normal subjects. Within subject CV of 20 % for S_I and within subject CV of 25 % for S_G have been reported.[11] This confirms the logical expectation that the modified protocol improves reproducibility of insulin sensitivity but not reproducibility of glucose effectiveness. Ferrari *et al*.[25] also reported highly reproducible estimates of insulin sensitivity with the tolbutamide-modified IVGTT using the unlabelled (one compartment) minimal model in normal subjects.

The critical question is the performance of these models in impaired glucose tolerance in various diabetes states and especially in Type 2 (non-insulin dependent) diabetes mellitus (DM). Current evidence suggests that the modification of IVGTT by insulin is essential for a good performance in Type 2 DM. The insulin-modified unlabelled IVGTT has been successfully employed and a good comparison with glucose derived clamp indices was obtained.[26] Avogaro *et al*.[27] documented an improved precision of estimates of insulin sensitivity in Type 2 DM using labelled glucose. Whether the improvement justifies the use of labelled glucose is difficult to assess but the ability of the labelled models to discriminate between glucose uptake and production may influence the decision in certain applications, e.g. in the assessment of the mode of action of new compounds. The performance of the two compartment model in Type 2 DM has not been reported. An extrapolation from the present study suggests that reproducibility and precision should be comparable with the labelled (one compartment) model.[27]

Comparability of Parameters of Glucose Kinetics

Insulin sensitivity S^*_I estimated by the one compartment (minimal) model was found to be strongly linearly related to insulin sensitivity S^*_{Ib} estimated by the two compartment model. However, no significant correlation was found between glucose effectiveness S^*_G estimated by the labelled one compartment model and plasma glucose clearance *PCR* estimated by the labelled two compartment model. The two indices *PCR* and S^*_G were expected to be related as the former represents the absolute and the latter fractional plasma clearance rate at fasting conditions.

It is not possible to compare directly indices of the

Table 2. Comparability and reproducibility of (1) insulin sensitivity (S^*_I and S_I) and glucose effectiveness (S^*_G and S_G) based on the labelled and unlabelled one compartment model, and (2) insulin sensitivity (S^*_{Ib}), plasma clearance rate (*PCR*), fasting hepatic glucose output (HGO_b), and cumulative hepatic glucose output (HGO_{0-240}) based on the labelled two compartment model of IVGTT. Mean ± SE values are reported

	One compartment model				Two compartment model			
	S^*_I (10^{-4} min^{-1} per mU l^{-1})	S_I (10^{-4} min^{-1} per mU l^{-1})	S^*_G (/min)	S_G (/min)	S^*_{Ib} (ml kg^{-1} min^{-1} per mU l^{-1})	*PCR* (ml kg^{-1} min^{-1})	HGO_b (mg kg^{-1} min^{-1})	HGO_{0-240} (mg kg^{-1} 240 min^{-1})
Day 1	13.17 ± 2.08	7.18 ± 0.96	0.0093 ± 0.0004	0.0308 ± 0.0047	0.317 ± 0.055	2.02 ± 0.16	2.01 ± 0.20	403 ± 32
Day 7	12.91 ± 1.83	8.90 ± 0.89	0.0085 ± 0.0006	0.0305 ± 0.0053	0.289 ± 0.038	1.93 ± 0.17	1.91 ± 0.19	403 ± 32
Mean (day 1 and 7)	13.04 ± 1.89	7.80 ± 0.64	0.0089 ± 0.0005	0.0307 ± 0.0044	0.303 ± 0.046	1.98 ± 0.15	1.96 ± 0.18	403 ± 31
Within subject CV	17%	38%[a]	13%	27%[b]	19%	16%	15%	7%
Within subject variation as % of total variation	13%	100%[a]	41%	27%[b]	14%	34%	22%	8%
95% range for difference between duplicate measurements in one individual	± 7.14	± 12.23[a]	± 0.0036	± 0.0266[b]	± 0.18	± 0.99	± 0.91	± 90

[a]Five subjects who provided estimate of S_I with acceptable precision on both occasions contributed to the reproducibility analysis.
[b]Nine subjects who provided estimate of S_G with acceptable precision on both occasions contributed to the reproducibility analysis.

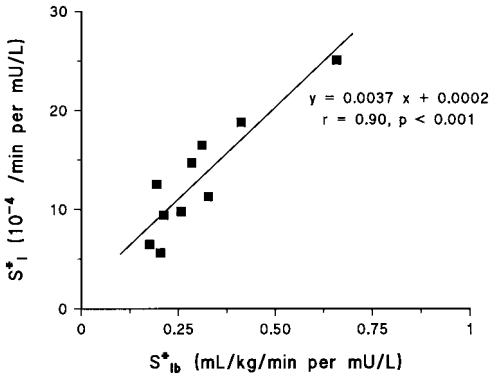

Figure 4. Relationship between insulin sensitivity S^*_{Ib} estimated by the two compartment model and insulin sensitivity S^*_I estimated by the (one compartment) minimal model. Means of duplicate measurements are plotted

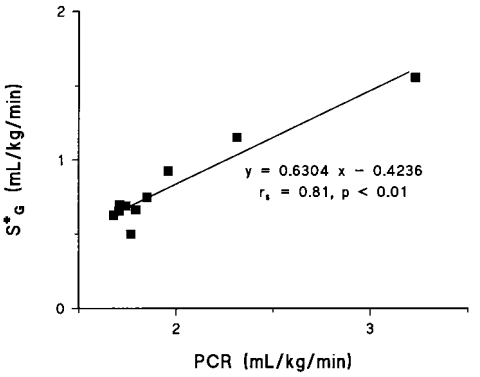

Figure 5. Relationship between plasma clearance rate *PCR* estimated by the labelled two compartment model and glucose effectiveness S^*_G estimated by the labelled one compartment minimal model. S^*_G was represented in the same units as *PCR* by multiplying glucose effectiveness by individual estimates of the plasma distribution volume obtained from the two compartment model. Means of duplicate measurements are plotted

one compartment and two compartment models as they are expressed in different units. The labelled two compartment model provides an estimate of the glucose distribution volume. Assuming this to be a correct estimate of the distribution volume for the one compartment model, it is possible to express S_I and S^*_I in the same units as S^*_{Ib} by multiplying the former two indices by an individual estimate of the distribution volume. These calculations showed that both S^*_I (0.120 ± 0.025 ml kg^{-1} min^{-1} per mU l^{-1}) and S_I (0.069 ± 0.008 ml kg^{-1} min^{-1} per mU l^{-1}) were significantly lower than S^*_{Ib} ($p < 0.01$, paired *t*-test, mean of duplicate estimates). The mean underestimation (considering the two compartment model as gold standard) was about two-to-three fold for S^*_I, and four-to-five fold for S_I. This is in agreement with theoretical calculations by Caumo *et al.*[28] who clearly demonstrated that the undermodelling (using one rather than two compartments) of glucose kinetics is expected to result in underestimation of insulin sensitivity indices. Saad *et al.*[29] similarly showed that S_I was 60 % lower in normal subjects than estimated from glucose clamp.

Using a similar approach, S_G and S^*_G can also be converted to the same units as the plasma clearance rate (*PCR*). We found that S_G was not significantly different from *PCR* (paired *t*-test) but the two indices were not related ($r_s = 0.18$, NS). S^*_G was significantly different from *PCR* ($p < 0.01$, paired *t*-test) but correlated with and linearly related to *PCR* ($r_s = 0.81$, $p < 0.01$; see Figure 5). These observations suggest that differences in the distribution volume between subjects could explain the lack of correlation between *PCR* and S^*_G when the latter is expressed in its original units (min^{-1}).

The observed range of S^*_I in the present study is higher but still compares well with the ranges of 6.6–15.2 and 2.23–8.20 $\times 10^{-4}$ min^{-1} per mU l^{-1} in two other studies.[8,13] The observed low fasting plasma insulin concentration during fasting normoglycaemia indicated that our subjects were highly insulin sensitive and this was supported by the low insulin response to the glucose stimulus (peak plasma insulin ~60 mU l^{-1}) with blood glucose concentration returning to the fasting level within ~60 min. Plasma clearance rate estimated by the labelled two compartment model compared well with the value 2.47 ± 0.12 ml kg^{-1} min^{-1} measured in normal subjects using the tracer dilution technique.[30]

The low insulin response partially explains the reduced precision of insulin sensitivity obtained from the unlabelled one compartment model. Due to lower absolute values of insulin sensitivity from the unlabelled than labelled one compartment model, the precision of S_I was lower than the precision of S^*_I.

That S^*_I was less than S_I was unexpected. Based on theoretical calculations, an opposite trend has been be present. No explanation is readily available. The same trend has been observed in other studies with radioactive[10] and stable-isotope[13] glucose tracers.

Hepatic Glucose Output

Compared to the (one compartment) minimal model, the two compartment model when using stable isotope tracers has the unique ability to provide plausible and accurate estimate of hepatic (or more accurately, endogenous) glucose output.[12,31] The basal hepatic glucose output of 1.96 ± 0.18 mg kg^{-1} min^{-1} estimated in the present study compares well with results obtained from 25 tracer dilution studies in normal subjects (mean ± SE 2.00 ± 0.05 mg kg^{-1} min^{-1}, range 1.43–2.40 mg kg^{-1} min^{-1}).[32]

The maximum suppression of *HGO* was lower than reported from similar studies[12,31] possibly due to lower plasma insulin in the present study. The maximum suppression was comparable with maximum suppression observed in hyperinsulinaemic euglycaemic clamp studies with portal delivery of insulin and plasma insulin concentration of ~30 mU l^{-1}.[33]

The total *HGO* demonstrated remarkable reproducibility. Visual inspection of Figure 3, however, showed differences between the two occasions indicating that

the dynamic profiles of *HGO* differed. The calculations of *HGO* are highly sensitive to measurement error (data not shown) and the error introduced by data analysis (primarily due to the choice of an incorrect regularization constant) probably underlies the observed differences. More research needs to be carried out in this area and new techniques are being developed.[34]

The present study did not make direct measurements of *HGO* insulin sensitivity by a glucose clamp technique. We cannot comment further about accuracy of estimated parameters.

In conclusion, the stable-label (one compartment) minimal model provides reproducible estimates of insulin sensitivity and glucose effectiveness. The stable-label two compartment model provides reproducible estimates of insulin sensitivity, plasma clearance rate, and basal and total hepatic glucose output in normal subjects. Insulin sensitivity indices estimated by the two labelled models are strongly linearly related.

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Appendix

Abbreviations

APE	atom per cent excess
D^*	dose of labelled glucose injected at time 0
D_i	proportion of molecules of RMW M+i in labelled glucose
F_{01}	constant component of glucose uptake (1 mg kg^{-1} min^{-1}) from the accessible pool of the two compartment model
g	plasma concentration of total (labelled and unlabelled) glucose
g^*	plasma concentration of labelled glucose
g_0, g^*_0	total and labelled plasma glucose concentration at time zero of the one compartment model, respectively
g_b	basal concentration of total plasma glucose
g_e	plasma concentration of glucose due to endogenous glucose production
g_x	plasma concentration of glucose due to unlabelled glucose in the exogenous glucose bolus
h	time-varying impulse response of glucose system
HGO	hepatic glucose output
HGO_{0-240}	total hepatic glucose output during the study (0–240 min)
HGO_b	basal hepatic glucose output
k_a, k_b	fractional rate constants associated with the remote insulin compartment of the two compartment model
k_{ij}	fractional rate constant from compartment j to compartment i of the two compartment model
k_p	fractional rate constant describing component of insulin-independent glucose uptake proportional to glucose concentration in the two compartment model
n	total number of molecules in the sample
n_D	number of molecules in the sample originating in labelled glucose
n_N	number of molecules in the sample originating in the endogenous glucose
N_i	proportion of molecules of RMW M+i in the endogenous glucose
i	plasma insulin concentration
i_b	basal plasma insulin concentration
p_1, p_2, p_3	parameters of the one compartment minimal model; *superscript denotes quantities of the stable-label model
PCR	plasma clearance rate of glucose
q^*_1, q^*_2	mass of glucose in accessible and non-accessible compartments of the two compartment model, respectively
r, r_N	peak isotope ratio and raw basal isotope peak ratio
S_G, S^*_G	glucose effectiveness of the unlabelled and labelled one compartment models, respectively
S_i	proportion of molecules of RMW M+i in the sample
S_I, S^*_I	insulin sensitivity of the unlabelled and labelled one compartment models, respectively
S^*_{Ib}	insulin sensitivity of the two compartment model
TTR, z	tracer-to-tracee ratio (both symbols are used)
V_1	distribution space of the accessible glucose compartment of the two compartment model
x	insulin action in the remote insulin compartment

Calculating TTR

TTR was calculated from the isotope (area) ratio employing the method given by Rosenblatt *et al.*[16] The raw measurement (isotope ratio) r, M+2/M+0, in the mixture of tracer and tracee (sample) can be written as

$$r = \frac{S_2}{S_0} = \frac{S_2 n}{S_0 n} = \frac{n_N N_2 + n_D D_2}{n_N N_0 + n_D D_0} = \frac{\frac{N_2}{N_0} + \frac{n_D D_2}{n_N N_0}}{1 + \frac{n_D D_0}{n_N N_0}} = \frac{r_N + z\frac{D_2}{N_0}}{1 + z\frac{D_0}{N_0}} \quad \text{(A1)}$$

where S_i represents the proportion of molecules of RMW M+i in the sample, n is the total number of molecules in the sample, N_i is the proportion of molecules of RMW M+i in the endogenous glucose (tracee), D_i is the proportion of molecules of RMW M+i in 6,6-^{2}H-glucose (tracer), n_N is the number of molecules in the mixture originating in the endogenous glucose, n_D is the number of molecules in the mixture originating in 6,6-^{2}H-glucose, z is TTR of 6,6-^{2}H-glucose, and r_N is the raw basal measurement (isotope ratio) M+2/M+0 (the ratio of the most abundant isotopemer in 6,6-^{2}H-glucose to the most abundant isotopemer in the endogenous glucose) in the basal sample. It follows by definition that $z = \frac{n_D}{n_N}$ and $r_N = \frac{N_2}{N_0}$. Solving equation (A1) for z, we obtain

$$z = \frac{r - r_N}{\frac{D_2}{N_0} - \frac{D_0}{N_0} r}. \quad \text{(A2)}$$

Values D_i and N_i were calculated from the natural abundance of atoms in the monitored fragment $C_{12}H_{19}O_7B_2$ (M=297) employing the knowledge on the atomic purity of 6,6-^{2}H-glucose (APE=98 %). The values were calculated as N_0 = 60.0594, D_0 = 4.0429, D_2 = 58.0243 %, and were employed to derive equation (2) from equation (A2).

Unlabelled Minimal Model of Glucose Disappearance (One Compartment Model)

The unlabelled minimal model of glucose disappearance is described by two differential equations

$$dg(t)/dt = -[p_1 + x(t)]g(t) + p_1 g_b \quad g(0) = g_0 \quad \text{(A3)}$$

$$dx(t)/dt = -p_2 x(t) + p_3[i(t) - i_b] \quad x(0) = 0 \quad \text{(A4)}$$

where $g(t)$ is plasma concentration of total (labelled and unlabelled) glucose, $i(t)$ is plasma insulin concentration, $x(t)$ is a variable associated with the remote insulin compartment, g_b is basal glucose concentration, i_b is basal insulin concentration, and p_1, p_2, p_3 and g_0 are model parameters. Insulin sensitivity is defined as a ratio $S_I = p_3/p_2$, glucose effectiveness as $S_G = p_1$.

Stable-Label Minimal Model of Glucose Disappearance (One Compartment Model)

The stable-label minimal model of glucose disappearance consists of two differential equations

$$dg^*(t)/dt = -[p^*_1 + x(t)]g^*(t) \quad g^*(0) = g^*_0 \quad \text{(A5)}$$

$$dx(t)/dt = -p^*_2 x(t) + p^*_3[i(t) - i_b] \quad x(0) = 0 \quad \text{(A6)}$$

where $g^*(t)$ is plasma concentration of 6,6-^{2}H-glucose, p^*_1, p^*_2, p^*_3 and g^*_0 are model parameters. Insulin sensitivity is defined as a ratio $S^*_I = p^*_3/p^*_2$, glucose effectiveness as $S^*_G = p^*_1$.

Stable-Label Two Compartment Model of Glucose Disappearance (Two Compartment Model)

The stable-label two compartment model of glucose disappearance during an IVGTT is described by a set of differential equations

$$dq^*_1(t)/dt = -[k_p + F_{01}/V_1/g(t) + k_{21}] \quad q^*_1(t) + k_{12}q^*_2(t) \quad q^*_1(0) = D^* \quad \text{(A7)}$$

$$dq^*_2(t)/dt = -[k_{02} + x(t) + k_{12}]q^*_2(t) + k_{21}q^*_1(t) \quad q^*_2(0) = 0 \quad \text{(A8)}$$

$$dx(t)/dt = -k_b x(t) + k_a[i(t) - i_b] \quad x(0) = 0 \quad \text{(A9)}$$

$$g^*(t) = q^*_1(t)/V_1 \quad \text{(A10)}$$

where q^*_1(t) and q^*_2(t) are masses of the tracer glucose in the two compartments, V_1 is the volume of the accessible compartment, k_p is the proportional term of glucose disposal, k_{21}, k_{12}, and k_{02} are fractional rate parameters, k_a and k_b have similar meaning as p^*_3 and p^*_2 of the one-compartment stable-label minimal model, F_{01} is the constant component of glucose uptake (fixed at 1 mg kg^{-1} min^{-1}[35]), and D^* is the administered dose of the tracer glucose. The proportional term of glucose disposal k_p is constrained to produce insulin-independent utilization three times higher than the insulin-dependent utilization at the basal glucose concentration (g_b) and the basal insulin concentration (i_b)

$$k_p = 3k_{21}k_{02}/(k_{02} + k_{12}) - F_{01}/(V_1 g_b) \quad \text{(A11)}$$

guaranteeing theoretical identifiability of the model.[12]

Basal clearance rate *PCR*, fasting hepatic glucose output HGO_b, and insulin sensitivity index S^*_{Ib} ($S^*_{Ib} = \partial PCR/\partial i \mid i = i_b$) are calculated as

$$PCR = F_{01}/g_b + V_1 k_p + V_1 k_{21} k_{02}/(k_{02} + k_{12}) \quad \text{(A12)}$$

$$HGO_b = g_b PCR \quad \text{(A13)}$$

$$S^*_{Ib} = V_1 k_{21} k_{12} k_a/(k_b(k_{02} + k_{12})^2) \quad \text{(A14)}$$

Hepatic Glucose Output

Hepatic glucose output was estimated using the labelled two compartment model with reconstruction of the input

function.[12] The plasma glucose component due to hepatic glucose output ($g_e(t)$, endogenous glucose component)[18,19] was calculated prior to calculating HGO. The total glucose concentration $g(t)$ contains three components, the endogenous glucose component $g_e(t)$, the unlabelled exogenous component $g_x(t)$, and the labelled exogenous component $g^*(t)$,

$$g(t) = g_e(t) + g_x(t) + g^*(t) \quad \text{(A15)}$$

The last two components are due to the exogenous glucose injection. They are fixed at a ratio E, $E = g^*(t)/g_x(t)$, which is given by the enrichment of the glucose bolus administered at the start of IVGTT. Solving equation (A15) for $g_e(t)$ we obtain

$$g_e(t) = g(t) - g^*(t)\,\frac{1+E}{E} \quad \text{(A16)}$$

The calculation of the endogenous glucose component is model-independent and only an assumption about the isotopic indistinguishability is made.[36]

The relation between $g_e(t)$ and hepatic glucose output $HGO(t)$ can be described by the integral equation

$$g_e(t) = \int_{-\infty}^{t} h(t,\tau)\,HGO(\tau)\,d\tau \quad \text{(A17)}$$

where $h(t,\tau)$ is the (time-variant) unit impulse response of the unlabelled glucose system.

The impulse response $h(t,\tau)$ was defined as a sum of two exponentials (a function of τ) for t between two sampling points, i.e. a piecewise linearization at the sampling points of the two compartment model was adopted,

$$h(t,\tau) = h_i(\tau) = A_{1,i}e^{-\lambda_{i,1}\tau} + A_{2,i}e^{-\lambda_{2,i}\tau} \text{ for } t_i \le t < t_{i+1}, \quad \text{(A18)}$$

where constants $A_{i,1}$, $A_{i,2}$, $\lambda_{i,1}$, and $\lambda_{i,1}$ were determined from parameters of the two compartment model, plasma glucose concentration, and predicted remote insulin (equation A9). Equations (A7) and (A8) were solved analytically for plasma glucose $g(t_i)$ and remote insulin $x(t_i)$ and the unit impulse response of tracer glucose system $g^*(t)$ (equation A10) expressed as a sum of two exponentials. The impulse response of the tracee glucose system is identical to the impulse response of the tracer glucose system assuming tracer indistinguishability.

The integral equation (A17) was solved using a regularization method with the regularization component consisting of the norm of second differences[12,37] to provide (piecewise constant) $HGO(t)$. The regularization coefficient which defines the amount of smoothing adopted by the regularization method was chosen individually, based on the distribution of residuals. To avoid the effect of smoothing on the hepatic glucose output at time 0 min, $HGO(0)$ was calculated as $HGO(0) = HGO_b$.